Ethanol induced modification of *m*-xylene toxicokinetics in humans

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Abstract

This study was undertaken to determine whether previous subacute treatment with ethanol could modify the kinetics of *m*-xylene in humans. A group of six volunteers was exposed twice to either 100 or 400 ppm of *m*-xylene during two hours (between 0800 and 1000). Ethanol was given orally in the early evening on each of two consecutive days before exposures (total ethanol intake of 137 g). Such ethanol pretreatment affected the kinetics of *m*-xylene but only at the high exposure (400 ppm). The modifications were: (1) decreased concentration of *m*-xylene in blood and alveolar air during and after exposure; (2) increased urinary excretion of m-methylhippuric acid at the end of exposure. Ethanol treatment also enhanced the elimination of antipyrine in saliva. Overall, this study showed that the effect of enzyme induction on the metabolism of *m*-xylene, after ethanol ingestion, depends on the exposure concentration and is not likely to occur as long as the exposure concentrations remain under the current maximum allowable concentration (100 ppm) in the workplace.

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The effects of ethanol on the disposition of foreign compounds depend on the presence of ethanol in the body and on the pattern of ethanol consumption. During acute ingestion and shortly thereafter, when ethanol still persists in blood, the biotransformation of other xenobiotics is inhibited. Several authors have reported examples of such acute inhibitory metabolic interaction between ethanol and several solvents in humans.[1–7] In most cases, the metabolic interaction has resulted in the following kinetic modifications: (1) increased concentration of solvents in blood and alveolar air, (2) decreased urinary metabolite excretion. On the other hand, once the ethanol has disappeared from the body, the biotransformation of xenobiotics is stimulated leading to opposite kinetic effects; such stimulation has been found in rats and was more noticeable when ethanol was ingested in a repetitive fashion.[8 9] This biotransformation stimulating effect of ethanol disappeared almost completely after one day without ingestion of ethanol.[8] Despite evidence for ethanol induced biotransformation of solvents in animals, no experimental data are available for humans.

Chronic ethanol consumption increases the activity of the microsomal cytochrome *P*-450, inducing a specific isoform, *P*-450IIE1,[10] which plays an important part in the oxidation of organic solvents[11] including *m*-xylene.[12] Xylenes (*o*, *m*, and *p*-isomers) are among a group of industrial solvents that are extensively used. They are found in gasoline and petroleum solvents and they have various industrial applications such as in painting, degreasing, and coating. They are also used in the production of drugs, dyes, and insecticides.[13] Xylene isomers are primarily metabolised to corresponding methylbenzoic acids, which are conjugated with glycine and excreted as methylhippuric acids in urine.

As ethanol ingestion has been reported to induce cytochrome *P*-450IIE1 in humans[14] there is a possibility that drinking of alcoholic beverages affects the overall metabolism of a wide variety of foreign compounds such as organic solvents, including xylenes, to which workers are occupationally exposed. It remains unclear, however, whether enzyme induction due to regular ethanol consumption accelerates the metabolism of organic solvents in industrial workers.

The purpose of this study was to investigate the effect of subacute ethanol pretreatment on the kinetics of *m*-xylene in humans. Also, antipyrine was used as a probe to investigate the activity of the mixed function oxydase system of the liver before and after ethanol pretreatment.

Subjects and methods

SUBJECTS

Six non-smoking adults, three women (age 27 to 56 years; weight 48 to 58 kg) and three men (age 28 to 57 years; weight 73 to 77 kg) who had not been occupationally exposed to solvents volunteered for the study. They were given a detailed medical examination, including electrocardiography, electroencephalography, and relevant haematological and biochemical tests.

EXPOSURE CHAMBER

Exposures to *m*-xylene were carried out in a dynamic, environment controlled exposure chamber measuring 18·1 m.^{3} The air flow was set at 2·5 m^3/min. *m*-Xylene atmospheres were generated into the chamber by introduction of the solvent with a high performance liquid chromatography (HPLC) pump

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(Varian Canada Co) into a 1 litre, three necked, round bottomed flask. Here it was mixed with clean compressed air and then swept by aspiration into the inlet duct of the chamber. During exposures, the concentration of *m*-xylene in the chamber was monitored by gas chromatographic analysis of air samples that were withdrawn from the chamber at 30 minute intervals. The chromatography system consisted of an HP-5890 gas chromatograph (Hewett Packard Co) linked with an automatic 10 port air sampling valve (Valco Instrument Co). The analyses were performed with a 180 cm x 2 mm (inside diameter) glass column packed with GP 5% SP-1200/1·75% Bentone 34 on 100/120 Supelcoport (Supelco Canada Co). The column temperature was 80°C, and that of the injection port and detector flame ionisation was set at 110°C. Solvent concentration in air was continuously monitored by infrared spectrophotometry (Miran 1-A, Foxboro Co). During the experiment, the variation in the atmospheric concentration of *m*-xylene never exceeded ±3% of the target concentrations (100 and 400 ppm).

EXPERIMENTAL DESIGN

The volunteers were exposed on two different occasions to either 100 ppm (434 mg/m^3) or 400 ppm (1736 mg/m^3) of *m*-xylene for two hours (0800–1000). On one of these occasions the volunteers ingested one bottle of white or red wine (725 ml of 12% v/v ethanol content) at dinner in the early evening on each of two consecutive days preceding the day of exposure (total ethanol intake of 137 g). On the two other occasions, the volunteers were instructed to avoid any alcoholic beverage or any medication during the four days before exposure. Before their entry into the chamber the volunteers were asked to empty their bladders.

m-XYLENE IN BLOOD AND ALVEOLAR AIR

At predetermined intervals, the concentration of *m*-xylene was measured during and up to four hours after exposure. Blood samples were taken from the antecubital vein, transferred into hypovials, and kept at 4°C until analysis. Standards were prepared by adding known amounts of *m*-xylene to control blood on the day of exposure. Standard and test blood samples were analysed by gas chromatography with the head space method.[15]

Alveolar (end tidal) air was gathered into a 3 litre gas sampling bag (Tedlar bag, SKC Co) immediately before the collection of blood and analysed within 30 minutes of sample collection. The concentration of *m*-xylene in head space gas (blood) and alveolar air was measured under the same chromatographic conditions that were used to monitor the concentration of *m*-xylene in the exposure chamber.

URINARY EXCRETION OF *m*-METHYLHIPPURIC ACID

Samples of urine were collected before the onset of exposure, at the end of exposure, and at two hour intervals during the next six hours. The urinary volume was recorded and an aliquot of each sample was transferred to a plastic tube and kept at −20°C until analysis. The content of *m*-methylhippuric acid was determined by HPLC according to the method described by Tardif *et al.*[16] The creatinine content of each urine sample was also measured by a method based on the Jaffé reaction with picric acid.

ANTIPYRINE ELIMINATION

In another set of experiments, the influence of ethanol on antipyrine elimination was investigated. The same experimental design as that described earlier for ethanol ingestion was used. Antipyrine was purchased from Aldrich Co (Milwaukee, WI) and was given orally (14·3 mg/kg) with 200 ml of tap water in the morning (0800). Samples of saliva were collected from ethanol pretreated and non-pretreated subjects at 0, 1, 2, 4, 7, and 10 hours after dosing. Antipyrine content in saliva was determined by reverse phase HPLC with ultraviolet detection (254 nm) according to the method reported by Metha *et al.*[17]

STATISTICS

A paired Students' *t* test was used to analyse the results (mean values (SD)) and the level of probability for significance was fixed at $p < 0.05$.

Results

CONCENTRATION OF *m*-XYLENE IN BLOOD

Figure 1 shows the effect of previous ethanol intake on the concentration of *m*-xylene in blood during and after exposure. These results show that previous ingestion of

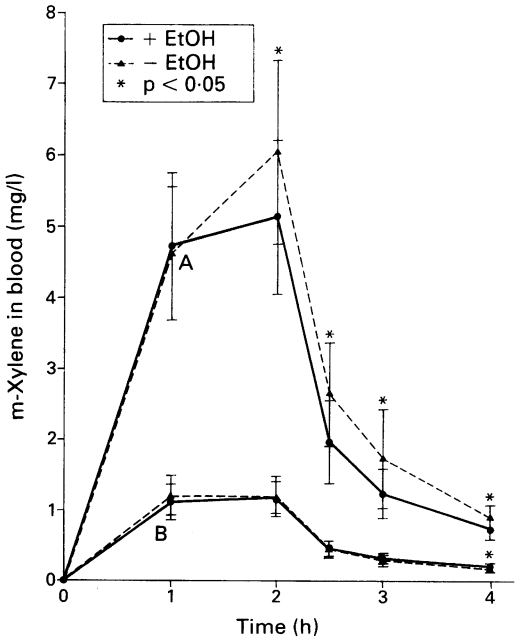

Figure 1 Effects of ethanol pretreatment on the time course concentration of m-*xylene in blood during and after exposure to* m-*xylene at 400 ppm (A) and 100 ppm (B) for two hours in volunteers. Vertical bars are SDs.*

ethanol resulted in lower concentrations of *m*-xylene in blood at the end of a two hour × 400 ppm exposure to *m*-xylene and during two hours thereafter. In the case of exposure to 100 ppm there were no such differences except for the last time of collection.

CONCENTRATION OF *m*-XYLENE IN ALVEOLAR AIR

In accordance with the findings in blood, only exposure at the higher concentration of *m*-xylene (400 ppm) resulted in repeatedly lower concentrations of the solvent in alveolar air (fig 2). For the 100 ppm scenario a small significant effect was apparent only at the end of the two hour exposure.

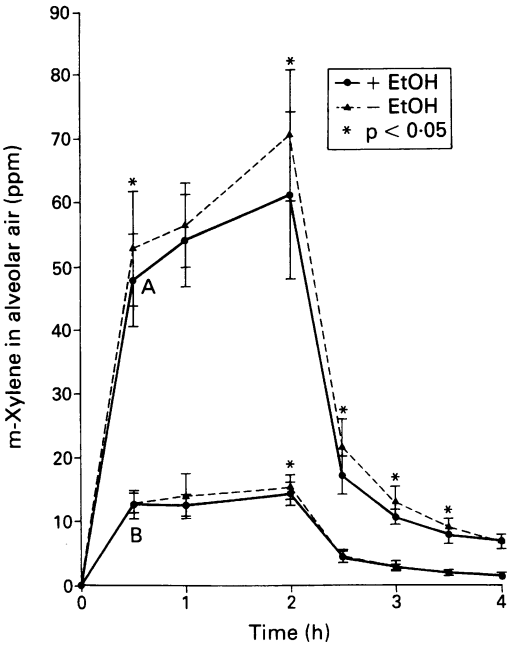

Figure 2 Effects of ethanol pretreatment on the time course concentration of m-*xylene in alveolar air during and after exposure to* m-*xylene at 400 ppm (A) and 100 ppm (B) for two hours in volunteers. Vertical bars are SDs.*

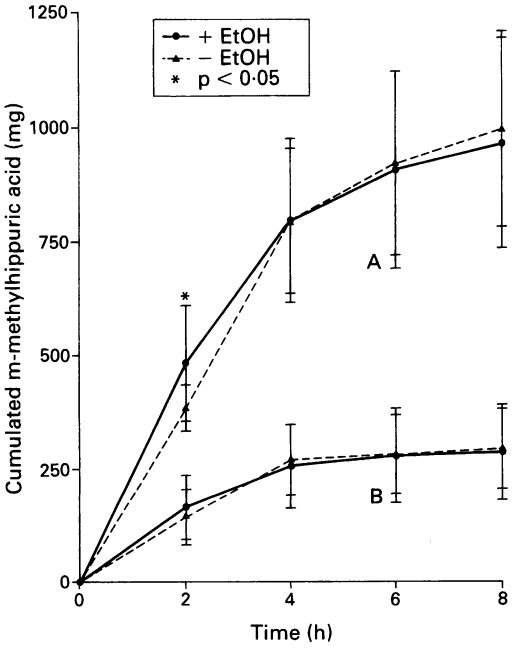

Figure 3 Effects of ethanol pretreatment on the cumulative urinary excretion of m-*methylhippuric acid during and after exposure to* m-*xylene at 400 ppm (A) and 100 ppm (B) for two hours in volunteers. Vertical bars are SDs.*

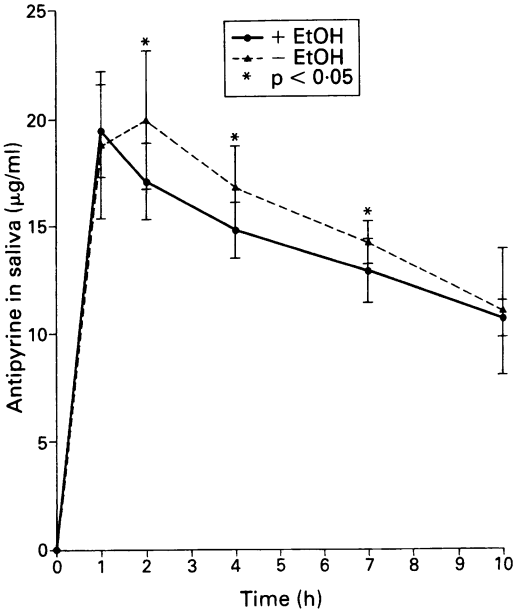

Figure 4 Effects of ethanol pretreatment on the time course concentration of antipyrine in saliva after giving antipyrine orally (14·3 mg/kg) to volunteers. Vertical bars are SDs.

URINARY EXCRETION OF *m*-METHYLHIPPURIC ACID

Figure 3 indicates that ethanol pretreatment resulted in a smaller effect on the urinary excretion of *m*-methylhippuric acid than on the concentration of *m*-xylene in blood and alveolar air. As expected, urinary excretion of *m*-methylhippuric acid was increased when the exposure concentration was 400 ppm; however, such an effect was apparent only at the end of the two hour exposure period.

ANTIPYRINE ELIMINATION IN SALIVA

Figure 4 illustrates the influence of previous ethanol intake on the elimination of antipyrine in saliva, which was slightly but significantly lower during the elimination phase.

Discussion

To our knowledge this is the first time that the metabolism enhancing effect of ethanol has been experimentally investigated in humans under controlled conditions. So far, the only study describing the effect of ethanol consumption in humans was conducted by Waldron *et al.*[3] These authors reported that workers exposed occupationally to toluene and who drank on a regular basis had lower concentrations of toluene in blood compared with workers who did not drink regularly. The present study suggests that ethanol pretreatment enhanced the metabolic clearance of *m*-xylene and antipyrine by inducing the enzyme(s) responsible for their respective biotransformation.

On the basis of in vitro and in vivo studies

ethanol pretreatment is known to enhance the metabolic disposition of many organic solvents in animals.[8 9 18] These experiments suggest that the turnover rate of cytochrome *P*-450IIE1 (ethanol inducible isozyme) might be rapid and that recently ingested ethanol might play an important part in enhancing the hepatic metabolism of such substances.

This study shows that previous ingestion of ethanol for two days is sufficient to modify the kinetics of *m*-xylene in humans. The effect, however, was limited to the higher concentration of exposure (400 ppm). This could be explained, in part, by the fact that *m*-xylene biotransformation is catalysed by a high capacity enzyme system. Thus at low concentration (100 ppm) *m*-xylene is probably completely metabolised while passing through the hepatic tissue (perfusion limited metabolism) and the effect of enzyme induction is only marginal. When the concentration of *m*-xylene is high, the metabolism is rather capacity limited and the influence of enzyme induction is more easily reflected in the overall disposition of *m*-xylene. Thus the effect of enzyme induction on the metabolism of *m*-xylene in vivo, after ethanol ingestion, depends on the exposure concentration.

The results of the present study support the hypothesis, established by a simulation study with a pharmacokinetic modelling approach[19] that enzyme induction can alter the kinetic profiles of highly metabolised compounds only at a high level of inhalation exposure. This hypothesis has also been supported by a recent animal experiment[20]: a three week treatment of rats with ethanol, which increased the in vitro metabolism of *m*-xylene about fivefold, accelerated the in vivo metabolism only at a high level of exposure (500 ppm). The ethanol treatment had no effect on the in vivo metabolism when rats were exposed to *m*-xylene at 50 or 100 ppm as evidenced by no significant change either in the *m*-xylene concentration in blood or in the excretion of *m*-methylhippuric acid in urine.

Antipyrine is one of the most extensively used probes to investigate the drug metabolising capacity of the liver, although the exact isoform(s) of cytochrome *P*-450 involved in its metabolism remain(s) unidentified. The antipyrine test is known to be highly sensitive to the enzyme inducing effect before and after environmental changes.[21 22] In the present study, similarly to what was seen for *m*-xylene, the disappearance of antipyrine in saliva was significantly enhanced by ethanol preatreatment, as reflected by a more rapid clearance from the blood. This suggests that ethanol inducible enzyme (*P*-450IIE1) may represent one of the three different cytochrome *P*-450 isozymes that are thought to be involved in the biotransformation of antipyrine.[23] There are few published reports on the metabolic induction effect of controlled treatment with ethanol on the metabolism of antipyrine in human volunteers. Moreover, there exist many discrepancies in the ethanol-antipyrine interaction. Vessel *et al*[24] reported that ethanol intake (1 ml/ kg/day for 21 days) decreased the mean half life of antipyrine by 22%. George *et al*[25] reported that ingestion of five alcoholic drinks during one week lowered the antipyrine clearance by 11%. Dossing and Andreasen[26] showed that ingestion of ethanol (0·73 g/kg as a loading dose and 0·15 g/kg/h for 12 hours) failed to alter the half life, distribution, or clearance of antipyrine when measured two days later. This lack of effect, however, could be due to the very short duration of the inductive effect of ethanol as seen in rats: the effect of enzyme induction by ethanol on the metabolism of various organic solvents completely disappeared one day after withdrawal of ethanol.[8] Concomitant intake of other drugs and smoking are further examples of factors that may influence the results of the antipyrine test.[23] In the present study, however, all volunteers were non-smokers and abstained from taking any drugs.

In conclusion the present study has shown that ingestion of one bottle of wine each night for two nights before a workshift may enhance the metabolism of *m*-xylene. Such an effect of ethanol consumption is seen, however, only at high exposure concentrations and does not affect the metabolism of *m*-xylene as long as the exposure concentrations remain under the current maximum allowable concentration (100 ppm) in the work environment.

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